Optimization of antioxidant extraction from *Solanum tuberosum* potato peel waste by surface response methodology

Isabel Rodríguez Amado[1], Daniel Franco[2], Marivel Sánchez[3], Carlos Zapata[4] & José Antonio Vázquez[1]*.

[1] Group of Recycling and Valorization of Waste Materials (REVAL)
Marine Research Institute (IIM-CSIC)
C/ Eduardo Cabello, 6. Vigo-36208. Galicia – Spain

[2] Meat Technology Center
C/ Galicia 4, Parque Tecnolóxico de Galícia, San Cibrán das Viñas. Ourense-32900. Galicia-Spain

[3] Department of Chemical Engineering
School of Engineering, University of Santiago de Compostela
Campus Sur. Santiago de Compostela-15786. Galicia-Spain

[4] Department of Genetics
CIBUS Building, University of Santiago de Compostela
Campus Sur. Santiago de Compostela-15786. Galicia-Spain

* Corresponding author e-mail: jvazquez@iim.csic.es
Tel: +34 986214469; fax: +34 986292762

Abstract

This study reports the optimised conditions (temperature, ethanol concentration and processing-time) for antioxidant extraction from potato peel (Agria variety) waste. At short extraction times (34 min), optimal yields of phenolic (TP) and flavonoid (Fv) compounds were reached at 89.9ºC and ethanol concentrations of 71.2% and 38.6%, respectively. The main phenolic compounds identified in the extracts were chlorogenic (Chl) and ferulic (Fer) acids. A significant positive correlation was found between antioxidant activity and *TP*, *Fv*, Fer and Chl responses. Potato peel extracts were able to stabilize soybean oil under accelerated oxidation conditions, minimising peroxide, totox and p-anisidine indices. The production of hexanal and 2-hexenal in soybean oil samples was maximal for extracts obtained at intermediate temperatures and ethanol concentrations. Our results demonstrate potato peel waste is a good source of antioxidants able to effectively limit oil oxidation, while contributing to the revalorisation of these agrifood by-products.

Keywords: Antioxidant extraction; potato peel waste; by-products upgrade; process optimization; response surface methodology.

1. Introduction

Lipid oxidation is a free radical chain reaction that causes major quality losses in the food industry. Lipid oxidation during preparation, processing and storage can lead to the development of rancidity and deterioration of oil and lipid containing food products (Gordon, 1991). The addition of synthetic antioxidants to these foods is one of the most efficient ways to reduce rancidity, minimise the production of toxic oxidation molecules and to extend the food's shelf life (Paiva-Martins, Correia, Felix, Ferreira, & Gordon, 2007). It is also well recognised that some antioxidant compounds can reduce the risk of human diseases (Gutteridge & Halliwell, 2010).

In contrast, other synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) have potential health hazards (Van Esch, 1996). Recently, the interest of consumers in the use of natural antioxidants has increased due to the belief that they will offer more health benefits than synthetic antioxidants. This scenario has led to the search for natural compounds with antioxidative properties (Wettasinghe & Shahidi, 1999) and indeed, different natural products have been investigated as a source of antioxidants (Moure et al., 2001). Special attention has been paid to wastes generated in the food industry, such as peel, wastewaters and seeds (Ayala-Zavala et al., 2011; Moure, Domínguez, & Parajó, 2006). In particular, phenolic compounds isolated from plants are recognised as the most promising group of molecules that help to prevent oxidation and maintain product quality (Shahidi & Wanasundara, 1997).

Potatoes (*Solanum tuberosum*) are one of the most widely consumed vegetables worldwide. Nowadays, potato consumption patterns are gradually changing from fresh to processed formulations (*e.g.*, mashed potatoes, chip potatoes, etc.) derived from fast food habits in developed countries. This has resulted in environmental problems associated with waste generated by such manufacturing processes. Potato peel waste (PPW) is the major waste from the potato processing industry and a potential source of functional and bioactive compounds, including not only antioxidants but also pigments, dietary fiber, vitamins and minerals (Teow et al., 2007).

Recently a variety of new methodologies for PPW management have been successfully applied, including pressurized liquid extraction (Singh & Saldaña, 2011; Wijngaard, Ballay, & Brunton, 2012) and microwave-assisted extraction (Singh et al., 2011; Wu et al., 2012). However, in practice, the idiosyncrasy of the agrifood industry and the lack of low-cost industrial equipment have limited the implementation of these technologies. The management of this by-product is even more complex since its phenolic substances are usually extracted using organic solvents (methanol, ethanol, acetone and ethyl acetate) with remarkable extraction capacity but high toxicity (Proestos & Komaitis, 2008). Alternatively, the use of ethanol has several advantages over other commonly used solvents. It is an environmentally-friendly solvent with high extraction efficiency and lower toxicity and cost.

One important aspect of the extraction of antioxidant compounds from plant materials is the selection of appropriate extraction conditions. It is not advisable to

generalize extraction conditions for all types of plant materials due to the diverse nature of natural antioxidants. Extraction processes are commonly optimised using one-factor-at-a-time approaches. However, it is well-known that optimal conditions or interactions between variables cannot be predicted with this methodology. Both problems can be overcome by employing response surface methodology (RSM), a tool used by many researchers to maximize or minimize various independent variables and predict optimal experimental conditions (Wardhani, Vázquez, & Pandiella, 2010; Anastacio & Carvalho, 2013).

This study aims to optimise the extraction of antioxidant compounds from potato peel waste and to evaluate its ability to limit oil oxidation as a potential alternative to commercial antioxidants. The extraction conditions (temperature, ethanol concentration and processing time) were studied to optimise the yields of phenolics (*TP*) and flavonoids (*Fv*), the *in vitro* antioxidant activity and the main phenolic compounds identified. Then, the ability of potato peel extracts to control lipid oxidation of soybean oil under accelerated conditions was studied. For this purpose peroxide (PV), anisidine (AV) and totox (TV) values, tiobarbituric acid reactive substances (TBARs), conjugated dienes and volatile compounds were determined as indices of lipid oxidation.

2. Materials and Methods

2.1. Chemicals

All the chemicals and reagents were of analytical grade. Folin–Ciocalteu phenol reagent and ascorbic acid were from Fluka (Steinheim, Germany). 1,1-Diphenyl-2-

122 picryhydrazyl (DPPH) radical, gallic acid (GA), protocatechuic acid, caffeic acid,
123 ferulic acid, sinapic acid, chlorogenic acid and p-coumaric acid were from Sigma-
124 Aldrich (St. Louis, MO). D-glucose was purchased from Panreac (Spain)
125
126 *2.2. Plant material, substrate preparation, chemical analysis and experimental*
127 *procedure*
128 Potato peel was obtained by abrasion in a mechanical peeler (Sammic PPC-6,
129 Azkoitia, Gipuzkoa, Spain) of Agria variety potatoes. Peel samples were
130 immediately vacuum packed and stored in the dark at -20 ºC in order to prevent
131 microbial spoilage and oxidation. Peel samples were then lyophilised and milled
132 using a laboratory batch mill to obtain powder peels (PP). The chemical
133 composition of PP was assessed in triplicate by analysing crude protein, ashes,
134 moisture, fat, total soluble sugar and total carbohydrate content. Total nitrogen
135 content was determined according to the Kjeldahl method and crude protein
136 content calculated as total nitrogen multiplied by 6.25. Ashes were obtained by
137 calcination at 600 ºC in a muffle furnace and moisture content determined after
138 heating at 105 ºC in an oven until constant weight. Fat content was measured by
139 gravimetric difference after liquid-solid extraction in a soxhlet extractor using
140 hexane as solvent. Total soluble sugars were quantified according to the method of
141 Dubois, Gilles, Hamilton, Rebers, and Smith (1956) using D-glucose as a standard.
142 Finally, the total carbohydrate content was determined by the difference between
143 total weight (subtracting protein, fat and ash) and moisture content.
144
145 The extraction of antioxidant compounds from PP samples was carried out using
146 the hydroalcoholic solutions and temperatures defined in Table 1. Extractions were

147 performed at a solid to liquid ratio of 1:20 (w/v) in a controlled water bath under
148 high agitation conditions. After the time of extraction defined for each assayed
149 condition (Table 1), samples were filtrated through Whatman Nº1 filter paper and
150 final extracts (filtrates) were lyophilised for analysis. The extraction yield was
151 determined gravimetrically.
152
153 *2.3. Determination of total polyphenol content (TP)*
154 The total phenolic content of PP ethanolic extracts was determined based on the
155 method of Singleton, Orthofer, and Lamuela-Raventós (1999), using the Folin–
156 Ciocalteu Reagent (FCR) with gallic acid as a standard. 1 mL of sample or blank
157 was mixed with 100 µL of FCR and, after 5 min, 1 mL of a Na_2CO_3 solution (7%)
158 was added. After incubation for 1 h at room temperature, the absorbance was read
159 at 760 nm (PerkinElmer® Lambda 25 UV/Vis spectrophotometer, PerkinElmer Inc.,
160 Massachusetts, USA) in 1 cm cuvettes. Readings were compared with a standard
161 curve of gallic acid and the total phenolic content was expressed as mg of gallic
162 acid equivalent per g of freeze dried solid (mg GAE /g).
163
164 *2.4. Determination of total flavonoid content (Fv)*
165 The total flavonoid content was determined according to the method of Zhishen,
166 Mengcheng, and Jianming (1999), slightly modified. Briefly, 1 mL of extract was
167 diluted with 250 µL of distilled water and 75 µL of $NaNO_2$ (5%) and 150 µL of $AlCl_3$
168 (10%) were added. Both reagent additions were spaced by 6 and 5 min
169 respectively. Then, 500 µL of 1M NaOH were added and made up with distilled
170 water to 2.5 mL. After 30 min, the absorbance was read at 510 nm against reagent
171 blank containing ethanol/water solutions instead of sample. The total flavonoid

content was determined using a standard curve of catechin (0-100 mg/L).

2.5. Phytochemicals quantification by HPLC

Cinnamic and benzoic acids were determined by reversed phase HPLC using a Jasco LG-1580-04 gradient mixer, a PU-980 main pump and a UV variable wavelength detector UV-1575. The C18 column was a Kinetex 2.6 μm (2.4x10 mm, Phenomenex, Torrance (CA), USA). Gradient elution was performed with water acidified with 0.5% acetic acid (solvent A) and methanol (solvent B) as follows: 0-3 min, 95%A; 3-18.3 min up to 50%B; 18.3-24.4 min, up to 70% B; 24.4-27.4 95%A and 27.4-35 min, 95% A. The flow rate was 0.4 mL/min. The UV detection profile was performed at 280 nm to detect gallic acid up to min 7 and then 324 nm to detect other phenolic acids. The limit of quantification was 0.7 mg/L. Calibration curves were performed for gallic, chlorogenic, ferulic, caffeic and p-coumaric acids ranging from 0.7 to 40 mg/L in all cases with R^2 higher than 0.992. The repeatability was always high, for samples and calibrates, with coefficients of variation lower than 0.4%.

2.6. Determination of 1,1-Diphenyl-2-picryhydrazyl (DPPH) radical-scavenging capacity

The antioxidant activity was determined with DPPH as a free radical, using an adaptation to microplate of the method described by Brand-Williams, Cuvelier, and Berset (1995). Antioxidant solutions (10 μL) were added to 200 μL of a 60 μM solution of DPPH in ethanol. The decrease in absorbance was followed at 515 nm every 5 min in a in a ThermoFisher Scientific microplate reader until the reaction reached a plateau (1 h). Appropriate solvent blanks were run for each sample,

197 which were analysed in triplicate. The radical-scavenging activity (*RSA*) was
198 calculated as a percentage of DPPH discoloration, using the equation (Barreira,
199 Ferreira, Oliveira, and Pereira, 2008):
200
201 $$RSA = \frac{\left(A_{control}\right)_{t=60\text{min}} - \left(A_{sample}\right)_{t=60\text{min}}}{\left(A_{control}\right)_{t=60\text{min}}} \times 100 \quad (1)$$
202
203 where A_{sample} is the absorbance at 515 nm of the DPPH in the presence of the
204 sample and $A_{control}$ is the absorbance at 515 nm of the DPPH solution in its
205 absence.
206
207 *2.7. β-carotene bleaching assay*
208 The β-carotene (βC) bleaching assay described by Marco (1968) was modified for
209 use with microplates (Prieto, Amado, Vázquez, & Murado, 2012).
210 For measuring the antioxidant capacity, 50 µL of sample were mixed with 250 µL of
211 reagent in a 96 well microplate. Appropriate solvent blanks were run for each
212 sample, which were analysed in triplicate. Absorbance readings (470 nm) were
213 taken at regular intervals in a ThermoFisher Scientific microplate reader until β-
214 carotene was decoloured (about 2 h). The antioxidant activity coefficient (*AAC*)
215 was calculated as follows (Moure, Domínguez, & Parajó, 2006):
216
217 $$AAC = \frac{\left(A_{sample}\right)_{t=120\text{min}} - \left(A_{control}\right)_{t=120\text{min}}}{\left(A_{control}\right)_{t=0} - \left(A_{control}\right)_{t=120\text{min}}} \times 1000 \quad (2)$$
218

where A_{sample} and $A_{control}$ denote the absorbance at 470 nm of the β-carotene with and without the sample, respectively.

2.8. Oxidation stability of soybean oil under accelerated conditions

Each PP extract obtained from every experimental condition defined in Table 1 was individually added to a sample of refined soybean oil (SO) provided by Aceites Abril (San Cibrao das Viñas, Ourense, Spain). The composition of the soybean oil according to CODEX Stan 210 normative was acidity (0.04%), peroxide index (<1.2 meq O_2/kg), moisture (<0.01%) and impurities (<0.01%). The fatty acid profile in percentage was myristic (0.09), palmitic (10.8), palmitoleic (0.1), stearic (5.1), oleic (19.5), linoleic (48.2), linolenic (4.6), arachidic (0.4), eicosenoic (0.2) behenic (0.6) and lignoceric (0.3).

Samples of 20 mL soybean oil ($\approx$ 16 g; ρ=0.8 g/L) were mixed with 250 μL of PP ethanolic extracts. Oil samples were stored in glass containers at 60 ºC for 14 days. At the end of this period the following parameters were determined: peroxide value, p-anisidine value, TOTOX, tiobarbituric acid reactive substances (TBARs), conjugated dienes and volatile compounds.

2.9. Determination of peroxide value (PV)

Peroxide value was determined following the AOAC procedure (2007). Oil sample (0.5 g) was dissolved with 10 mL of trichloromethane. 15 mL of acetic acid and 1 mL of saturated aqueous solution of potassium iodide were then added. The sample was slightly shaken for 1 min and kept in the dark for 5 min. Once incubation was finished, 75 mL of distilled water was added and the sample was

vigorously shaken. Finally, liberated iodine was titrated with 0.01 N sodium thiosulfate in an automatic titrator. Peroxide value, expressed as milliequivalents O_2 kg^{-1}, was calculated according to the formula:

$$PV = \frac{V \times N \times 1000}{W} \quad (3)$$

where V is the volume (mL) of sodium thiosulphate consumed in the titration, N is the normality of the sodium thiosulfate solution and W is the sample weight (g). The final results of PV were normalised using the ratio between $PV_{samples}/PV_{control}$ (dimensionless).

2.10. Determination of p-anisidine value

Determination of p-anisidine value was carried out following an IUPAC method (1987). Oil samples (0.5-2 g) were dissolved in isooctane in a 25 mL volumetric flask. The sample was then reacted with p-anisidine solution in acetic acid (0.25% w/v) for 10 minutes to produce a colored complex. Absorbance with and without p-anisidine solution was measured at 350 nm and the parameter (AV) calculated according to the formula:

$$AV = \frac{25 \times \left[1.2 \times \left(E_b - E_a\right)\right]}{W} \quad (4)$$

where E_b is the net absorbance of the oil-solution, E_a is the net absorbance of the oil-anisidine-solution and W is the sample weight (g). The final results of AV were normalised using the ratio between $AV_{samples}/AV_{control}$ (dimensionless).

2.11. Determination of Totox value

The overall oxidation state of oil given by the Totox value was calculated according to the formula:

$$TV = AV + 2PV \quad (5)$$

2.12. Analysis of volatile compounds

The extraction of volatile compounds was performed using solid-phase microextraction (SPME). An SPME device (Supelco, Bellefonte, PA, USA) containing a fused-silica fibre (10 mm length) coated with a 50/30 μm thickness of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was used. For headspace SPME (HS-SPME) extraction, 0.36 g of each sample was used. The fibre, previously conditioned by heating in a gas chromatograph injection port at 270 °C for 60 min, was inserted into the sample vial and then exposed to headspace. Extractions were carried out in an oven at 60 ºC for 45 min, after sample equilibration for 15 min at the extraction temperature, ensuring a homogeneous temperature for sample and headspace. Once sampling was

285 finished, the fibre was withdrawn into the needle and transferred to the injection
286 port of the gas chromatograph–mass spectrometer (GC–MS) system.

287

288 A gas chromatograph 6890N (Agilent Technologies, Santa Clara, CA, USA)
289 equipped with a mass selective detector 5973N (Agilent Technologies) was used
290 with a DB-624 capillary column of 30 m× 0.25 mm id, 1.4 µm film thickness (J&W
291 Scientific, Folsom, CA, USA). The SPME fibre was desorbed and maintained in the
292 injection port at 260 °C for 8 min. The sample was injected in splitless mode.
293 Helium was used as a carrier gas with a linear velocity of 40 cm/s. The
294 temperature program was isothermal for 10 min at 40 ºC, raised to 200 ºC at a rate
295 of 5 ºC/min, and then raised to 250 ºC at a rate of 20 ºC/min, and held for 5 min:
296 total run time 49.5 min. Injector and detector temperatures were both set at 260 ºC.
297 The mass spectra were obtained using a mass selective detector working in
298 electronic impact at 70 eV, with a multiplier voltage of 1953 V and collecting data at
299 a rate of 6.34scans/s over the range *m*/*z* 40–300. Compounds were identified
300 comparing their mass spectra with those contained in the NIST05 (National
301 Institute of Standards and Technology, Gaithersburg, MD, USA) library (>80% of
302 coincidence) and/or by calculation of the retention index relative to a series of
303 standard alkanes (C5–C14) for calculating Kovats indices (Supelco) and matching
304 them with data reported in literature. The results for each volatile compound were
305 the mean value of three replicates and finally expressed in normalised form using
306 the ratio between $Volatile_{samples}/Volatile_{control}$ (dimensionless).

307

308 *2.13. Experimental design and statistical analysis*

The extraction of antioxidants as a function of temperature (*T*), ethanol concentration (*E*) and time-processing (*t*) was studied using a rotatable second order design with six replicates in the centre of the experimental domain. The conditions of the independent variables studied were: *T* in the range (25-90ºC), *E* in the range (20-100%) and *t* among (5-150 min). The encoding procedure of the variables was performed by the following formulas:

Codification	**Decodification**
$V_c=(V_n-V_0)/\Delta V_n$	$V_n= V_0+(\Delta V_n \times V_c)$

V_n: natural value in the centre of the domain
ΔV_n: increment of V_n per unit of V_c

Both expressions of the independent variables, codified and natural values, in each experimental run are summarized in Table 1.

Orthogonal least-squares calculation on factorial design data were used to obtain, by means of orthogonal least-squares calculation, empirical equations describing the different antioxidant activities or dependent variables (*R*), each one related to *T*, *E* and *t* effects. The general form of the polynomial equations is:

$$R = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2 \qquad (6)$$

where *R* represents the antioxidant response to be modelled; b_0 is the constant coefficient, b_i is the coefficient of linear effect, b_{ij} is the coefficient of interaction effect, b_{ii} the coefficients of squared effect, *n* is the number of variables and X_i and

331 X_j define the independent variables (*T*, *E* and *t*). The statistical significance of the
332 coefficients was verified by means of the Student t-test (α=0.05), goodness-of-fit
333 was established as the adjusted determination coefficient (R^2_{adj}) and the model
334 consistency by the Fisher F test (α=0.05) using the following mean squares ratios:
335
336

	the model is acceptable when
F1 = Model / Total error	$F1 \geq F_{den}^{num}$
F2 = (Model + Lack of fitting) / Model	$F2 \leq F_{den}^{num}$
F3 = Total error / Experimental error	$F3 \leq F_{den}^{num}$

337

338 F_{den}^{num} are the theoretical values to α=0.05 with the corresponding degrees of
339 freedom for numerator (num) and denominator (den). All fitting procedures,
340 coefficient estimates and statistical calculations were performed on a Microsoft
341 Excel spreadsheet.
342

343 **3. Results and Discussion**

344 *3.1. Proximate composition of powder peel (PP)*

345 The average (±SD) chemical composition of PP from cv. Agria, expressed as a
346 percentage of dry weight, was 6.47 ± 0.23% protein, 5.46 ± 0.17% ash, 0.92 ±
347 0.17% soluble sugar and 86.97 ± 0.43 for carbohydrates. The content of moisture
348 was 7.30 ± 0.23% of total weight. No fat was detected in the samples. PP
349 composition analysis gave lower values for protein and ashes than those
350 previously reported by Camire, Violette, Dougherty, and Laughlin (1997) in potato
351 peel, using abrasion and steam peeling procedures. These authors found that

chemical composition of PP (on dry weight basis) varied in ranges of 3.73-5.50%, 16.72-18.55%, 6.01-7.73%; 70-72%; for moisture, protein, ash and carbohydrate content, respectively. The chemical composition for other compounds was 6.55% moisture, 13.9% crude protein, 8.48% ashes, 13.0% crude fibre, and 56.2% carbohydrates.

3.2. Optimisation of antioxidant extraction

Several organic solvents have been previously studied for antioxidant extraction from potato waste obtaining different results. The higher extractions yields were generally obtained using methanol followed by ethanol (Mohdaly, Sarhan, Smetanska, & Mahmoud, 2010; Samarin, Poorazarang, Hematyar, & Elhamirad, 2012) whereas hexane and acetone yielded lower levels of these compounds (Samarin, Poorazarang, Hematyar, & Elhamirad, 2012). In this study, ethanol was selected as the extracting solvent because it has fewer restrictions for food applications. The combined effect of temperature, ethanol concentration and time of extraction was studied by means of surface response methodology.

The design and responses (experimental and predicted) of the 3-factor rotatable design are summarized in Table 1. Data from antioxidant extraction were converted into second-order polynomial equations as a function of three independent variables (*T*, *E* and *t*). Consequently, the polynomial model describing the correlation between the response and the variables followed the general form defined by equation [6] (Table 2). Second order equations accounted for more than 78% of the response variability, indicating that experimental data are in agreement with the responses predicted. These models can therefore be considered good

377 predictors for the extraction of antioxidants from potato peel waste in the range of
378 temperature, ethanol concentration and time of extraction studied.
379
380 Results of the multivariate analysis showed that the statistical significance of
381 coefficients was dependent on the response considered. For instance, all
382 parameters were significant (p<0.05) for extraction yield and flavonoid recovery,
383 while DPPH activity was only affected by *T* and *E* linear terms and the *E* quadratic
384 term. Figure 1 shows a selection of theoretical response surfaces corroborating the
385 observed variability in the responses. Thus, total polyphenol (TP) and flavonoid
386 recovery increased with increasing temperatures and at medium to high ethanol
387 concentrations. At short extraction times (34 min), optimal yields of phenolic (TP)
388 and flavonoid (Fv) compounds were reached at 89.9ºC and ethanol concentrations
389 of 71.2% and 38.6%, respectively. The highest DPPH scavenging activity is
390 achieved at 66.4% ethanol for increasing temperatures and at any time of
391 extraction (time had no effect on the recovery of radical-scavenging capacity).
392 Finally, antioxidant activity determined as β-carotene bleaching lead to a concave
393 surface with minima at 58ºC, 72% ethanol and 34 min of extraction. However, the
394 highest activity was observed at 89.9ºC and 20% ethanol.
395
396 The differences on the responses observed of antioxidant activity determined by
397 different *in vitro* methods are due to the fact that each assay quantifies various
398 phenolic acids or flavonoids with different antioxidant capacities depending on their
399 chemical structure (Pokorny, 2003). The improvement of extraction yields with
400 increasing temperature and ethanol concentration has been widely reported
401 (Wardhani, Vázquez, & Murado, 2010; Wijngaard, Ballay, & Brunton, 2012). This is

402 a result of the modification of the physical properties of the substrates, especially
403 viscosity and density, thereby improving the diffusivity of the solvent and the
404 solubility of antioxidants, resulting in an increase in the extraction yield (Herrero,
405 Martin-Alvarez, Señoráns, Cifuentes, & Ibañez, 2005).
406
407 Results from the literature and the present study on different polyphenol extraction
408 methods and their yields for several varieties of *Solanum tuberosum* are
409 summarised in Table 3. According to this information, methanol, ethanol and water
410 are the most commonly used solvents due to their polar nature that facilitates
411 solubilising polar compounds such as polyphenols. The extraction procedure is
412 also a key factor to be considered in the polyphenol recovery yield; pressurised
413 liquids and subcritical water methods are the most efficient (Singh & Saldaña,
414 2011; Wijngaard, Ballay, & Brunton, 2012). However, other factors such as potato
415 variety and solid/liquid ratio also have a significant effect on the polyphenol
416 extraction yields. In fact, the total polyphenol content showed a relationship with
417 potato peel colour according to the following sequence: red>brown>white, since
418 the presence of anthocyanins associated to red varieties contributes to higher TP
419 concentrations in comparison with white colour varieties. In Table 3, TP content in
420 Agria variety was expressed as 3.2-10.3 mg GAE/100 g dry peel in order to
421 standardise the units, as it is usual in the literature.
422
423 The validity of the design and polynomial models proposed is in agreement with
424 the findings of other authors using pressurized liquids (Wijngaard, Ballay, &
425 Brunton, 2012) and microwave-assisted extraction (Singh et al., 2011; Wu et al.,
426 2012). The similarity found in the DPPH response surface obtained in this study is

427 especially remarkable with that previously found by Wijngaard, Ballay, and Brunton
428 (2012), though the equation described by these authors did not show a significant
429 effect of extraction time.
430
431 *3.3. Characterisation of antioxidant compounds*
432 The two major phenolic compounds identified and quantified (Table 1) by HPLC in
433 the ethanolic extracts were chlorogenic (Chl) and ferulic (Fer) acids. Minor peaks of
434 gallic acid were also detected in some samples but most of them only showed
435 hydroxycinnamic acids. The theoretical response surfaces showing the influence of
436 the extraction conditions on both Chl and Fer contents are depicted in Figure 2. As
437 can be seen, the effect of extraction time was not significant for the recovery of Chl
438 and Fer from potato peel, and for chlorogenic acid production only ethanol
439 concentration was statistically relevant (Table 2). On the contrary, the highest
440 ferulic acid content is obtained at 72.8% ethanol for increasing temperature and
441 any time of extraction.
442
443 Chlorogenic and caffeic acids are the major phenolic compounds of potato peel
444 reported in the literature, but other phenolic acids such as gallic, ferulic, p-
445 hydroxybenzoic, p-coumaric and trans-o-hydroxycinnamic acids are also found
446 (Mohdaly, Hassanien, Mahmoud, Sarhan, & Smetanska, 2013). Deuber, Guignard,
447 Hoffmann, and Evers (2012) reported that chlorogenic acid and its isomers, neo-
448 and cryptochlorogenic acid, are the predominant compounds in potato peel. Also,
449 Nara, Miyoshi, Honma, and Koga (2006) found free chlorogenic and caffeic acids
450 form and ferulic acid in its bond structure in potato peel variety Toyoshiro. The
451 effect of solvent on the type of phenolic acid extraction was evidenced by the

452 results of Singh and Saldaña (2011) and Farvin, Grejsen, and Jacobsen (2012).
453 These authors extracted higher amounts of gallic acid using subcritical water
454 compared to methanol or ethanol. According to these authors, conventional
455 aqueous extracts also contained significantly (p<0.001) higher levels of gallic acid
456 when compared to the corresponding ethanol extracts.
457
458 The relationship of antioxidant activity (DPPH radical scavenging) with each
459 response variable (TP, Fv, Fer and Chl) was explored using the Pearson product-
460 moment correlation coefficient (r). Significant positive correlations were detected
461 between antioxidant activity and each response variable (TP, r= 0.73; p<0.001, Fv,
462 r= 0.68; p=0.001; Fer, r= 0.53; p<0.016, and Chl, r= 0.70; p<0.001). This finding
463 indicates that these phenolic compounds are responsible for the antioxidant
464 activity. The existence of this correlation has been previously described in the
465 literature. In fact, a significant positive correlation (r=0.43, p$\leqslant$0.05) between
466 polyphenolic compounds and their antioxidant activity was observed in peel
467 extracts from Marcy and Penta potato varieties (Mohdaly, Hassanien, Mahmoud,
468 Sarhan, & Smetanska, 2013). Nara, Miyoshi, Honma, and Koga (2006) reported
469 DPPH radical scavenging activities of chlorogenic and caffeic acids in potato peel
470 (7.87 and 3.95 μmol Trolox equiv/g dry matter, respectively), which accounted for
471 57% of total activity from potato peel (20.72 μmol Trolox equiv/g dry matter). These
472 authors concluded that antioxidant activity and the concentration of phenolic
473 compounds were closely related; chlorogenic and caffeic acids are important
474 components of free-form phenolics in potato peel. Wijngaard et al. (2012) also

475 reported total polyphenols and caffeic acid to be highly correlated with DPPH
476 activity (r= 0.82, p≤0.05; and r= 0.88, p≤0.01, respectively).
477
478 *3.4. Effect of extracts on soybean oil oxidation*
479 Oxidative stability tests were carried out under accelerated oxidation conditions
480 (60ºC, 15 days) because ambient conditions require an excessively lengthy
481 storage time. To analyse the antioxidant efficacies of PP extracts peroxide (*PV*),
482 anisidine (*AV*) and totox (TV) values were determined as indices of lipid oxidation
483 in soybean oil. Although conjugated dienes and thiobarbituric acid were also
484 quantified in ethanolic extracts following the methods of White (1995) and IUPAC
485 (1987), the empirical equations were not good predictors for these experimental
486 data and so they were excluded from the response surface analysis.
487
488 Nearly identical empirical equations were obtained for *PV* and *TV* values (Table 4);
489 the temperature of extraction was the only statistically significant variable affecting
490 both lipid oxidation profiles (Table 4). As can be seen in Figure 3, the highest
491 extraction temperatures lead to minimising *PV* values. By contrast, p-anisidine
492 production was also influenced by *T* and *E* quadratic terms of extraction (Table 4)
493 producing a response surface with a maximum for p-anisidine formation within the
494 experimental domain (45ºC and 60% ethanol). Nevertheless, conditions limiting oil
495 oxidation were high extraction temperature (minimises *PV*, *AV* and *TV* values) and
496 medium to high ethanol concentrations (minimises *AV* index). This agrees with the
497 process variables leading to the highest extraction of polyphenols and flavonoids
498 and antioxidant activities (Figure 1).

499

500 *PV* is a measure of the concentration of peroxides and hydroperoxides formed in
501 the initial stages of lipid oxidation and is one of the most utilised methods for the
502 measurement of oxidative rancidity in oils and fats. Since these compounds are
503 primary products of lipid peroxidation, *PV* index gives a clear indication of lipid
504 autoxidation. The utilisation of p-anisidine measurement to assess the potential of
505 natural antioxidants in vegetable oils under accelerated storage conditions is
506 generally accepted for further confirmation of these results, (Chatha, Anwar,
507 Manzoor, & Bajwa, 2006).

508

509 Oxidation indices ranged from 13.1 to 17.6, 293.0 to 380.2 and 139.6 to 182.1
510 meq O_2/kg oil for *AV*, *TV* and *PV*, obtaining maximum inhibition percentages
511 (calculated as 100x(1-(index value of sample/index value of control))) of 19.3, 22.4
512 and 22.8%, respectively. These results indicated that PP extracts obtained under
513 experimental conditions maximising antioxidant extraction and minimising oxidation
514 indices, were able to inhibit soybean oil oxidation. Suja et al. (2004) obtained
515 similar results with methanolic sesame extracts during the accelerated storage of
516 soybean oil (60ºC, 15 days). Although these authors did not optimise antioxidant
517 extraction, similar *PV* inhibition values (18.2 and 19.8%) were reported for sesame
518 extracts added at concentrations of 50 and 100 ppm to oil samples.

519

520 Several volatile compounds (VC) responsible for off flavours during soybean oil
521 storage are produced. These molecules represent secondary oxidation products,
522 resulting from the auto-oxidation of oleic, linoleic and linolenic acid. According to
523 mass spectra, 14 VC were identified and classified as 12 aldehydes (pentanal,

524 hexanal, 2-hexenal, 2,4-heptadienal, 2-octenal, nonanal, 2-nonenal, benzaldehyde-
525 3-ethyl, 2,4-nonadienal, 4-oxononanal, 2,4-decadienal and 2-dodecenal) and 2
526 ketones (3-octen-2-one, 3,5-octadien-2-one) (data not shown). Hexanal, pentanal
527 and nonanal were the predominant volatile compounds, followed by 2,4-
528 heptadienal and 3,5-octadien-2-one. The remaining volatile compounds were
529 minority, with concentrations below 30 UAx10^6/g oil. Mildner-Szkudlarz, Jelen,
530 Zawirska-Wajtasiak, and Wasowicz (2003) identified and quantified VC in soybean
531 oil stored at 60ºC for 5 days by headspace-solid phase microextraction. These
532 authors described 2-heptenal, hexanal and 2,4-heptadienal as the most
533 predominant VC in oxidized soybean oil. Steenson, Lee, and Min (2002) previously
534 reported hexanal as a lipid oxidation indicator in oil systems. Likewise Mildner-
535 Szkudlarz, Jelen, Zawirska-Wajtasiak, and Wasowicz (2003) reported that the
536 hexenal/nonanal ratio is important to the overall quality of soybean oil and Frankel
537 (1993) directly associated hexanal, 2-heptenal, 2-octenal and nonanal to the
538 overall rancidity of soybean oil.
539
540 Among the 14 compounds identified, pentanal, hexanal and 2-hexenal (Table 4)
541 were significantly affected by the conditions of antioxidant extraction defined in
542 Table 1, although the goodness-of-fit of the first two compounds was low. The
543 processing conditions (*T*, *E* and *t*) for the recovery of PP extracts that minimise the
544 production of such compounds were dependent on the molecule considered
545 (Figure 3). In general, the production of hexanal and 2-hexenal relative to control
546 oil was maximal for extracts obtained at intermediate temperature and ethanol
547 concentrations. These results confirm those observed for the p-anisidine index

548 (Figure 3). Therefore, both compounds appear to be responsible for the secondary
549 oxidation of soybean oil in these conditions.
550
551 **Conclusions**
552 In this study, an experimental design to look into the effect of three variables (time,
553 temperature and ethanol concentration) on antioxidant extraction from potato peel
554 waste was employed. In general, both increasing temperature and ethanol
555 concentrations lead to enhanced extraction yields that were slightly improved by
556 increasing the treatment time. The main phenolic compounds identified in PP
557 extracts were chlorogenic (Chl) and ferulic (Fer) acids, obtaining a significant
558 positive correlation between antioxidant activity and TP, Fv, Fer and Chl
559 responses.
560 Potato peel ethanolic extracts were able to stabilize soybean oil under accelerated
561 oxidation conditions, minimising PV, AV and TV indices at high temperature and
562 medium to high ethanol concentrations of extraction. According to mass spectra,
563 14 volatile compounds were identified being hexanal, pentanal and nonanal the
564 predominant in oxidized oil. Among them, hexanal and 2-hexenal might be
565 responsible for the increase in p-anisidine index (Figure 3) and therefore for the
566 secondary oxidation of soybean oil. The present study showed that potato peel is a
567 good source of antioxidants able to effectively limit oil oxidation. However, further
568 studies are required to confirm this hypothesis and extend the application of these
569 extracts to foods, such as meat and fish products where a complex mixture of
570 proteins, lipids, prooxidants and endogenous antioxidants are present.
571
572 **Acknowledgements**

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FIGURE CAPTIONS

Figure 1: Selection of the most relevant theoretical response surfaces describing the combined effects of the variables studied on antioxidant extraction from potato peel. TP: total polyphenols; DPPH: DPPH scavenging activity; Fv: total flavonoids.

Figure 2: Effect of variables studied on ferulic (Fer) and chlorogenic acid (Cl) aqueous extraction from potato peel.

Figure 3: Effect of PP extracts obtained in the conditions defined in Table 1 on lipid oxidation of soybean oil. *PV*: Peroxide value; *AV*: p-anisidine value; *TV*: Totox index. The independent variables used to obtain the extracts are written in real values.

TABLE CAPTIONS

Table 1: Independent variables in the response surface design with the corresponding experimental (R_{exp}) and predicted (R_p) results of ethanolic extraction. X_1: temperature (ºC); X_2: ethanol concentration (%) and X_3: extraction time (min). TP: total polyphenols; DPPH: DPPH scavenging activity; Fv: total flavonoids; Y: extraction yield; Fer: ferulic acid; Cl: chlorogenic acid. Natural values of experimental conditions are in brackets.

Table 2: Second order equations describing the antioxidant capacities studied, as a function of *T*, *E* and *t* (used in coded values according to criteria defined in Table 1). The coefficient of adjusted determination (R^2_{adj}) and F-values (F_1, F_2 and F_3) is also shown. S: significant; NS: non-significant.

Table 3: Extraction method and total phenolic (gallic acid equivalent) content in several potato peel varieties. db: dry basis.

Table 4: Equations describing the lipid oxidation responses measured regarding experimental conditions of *T*, *E* and *t* for antioxidant extraction from PP. Codification is according to the criteria defined in Table 1. The coefficient of adjusted determination (R^2_{adj}) and F-values (F_1, F_2 and F_3) is also shown. S: significant; NS: non-significant.

FIGURE 1

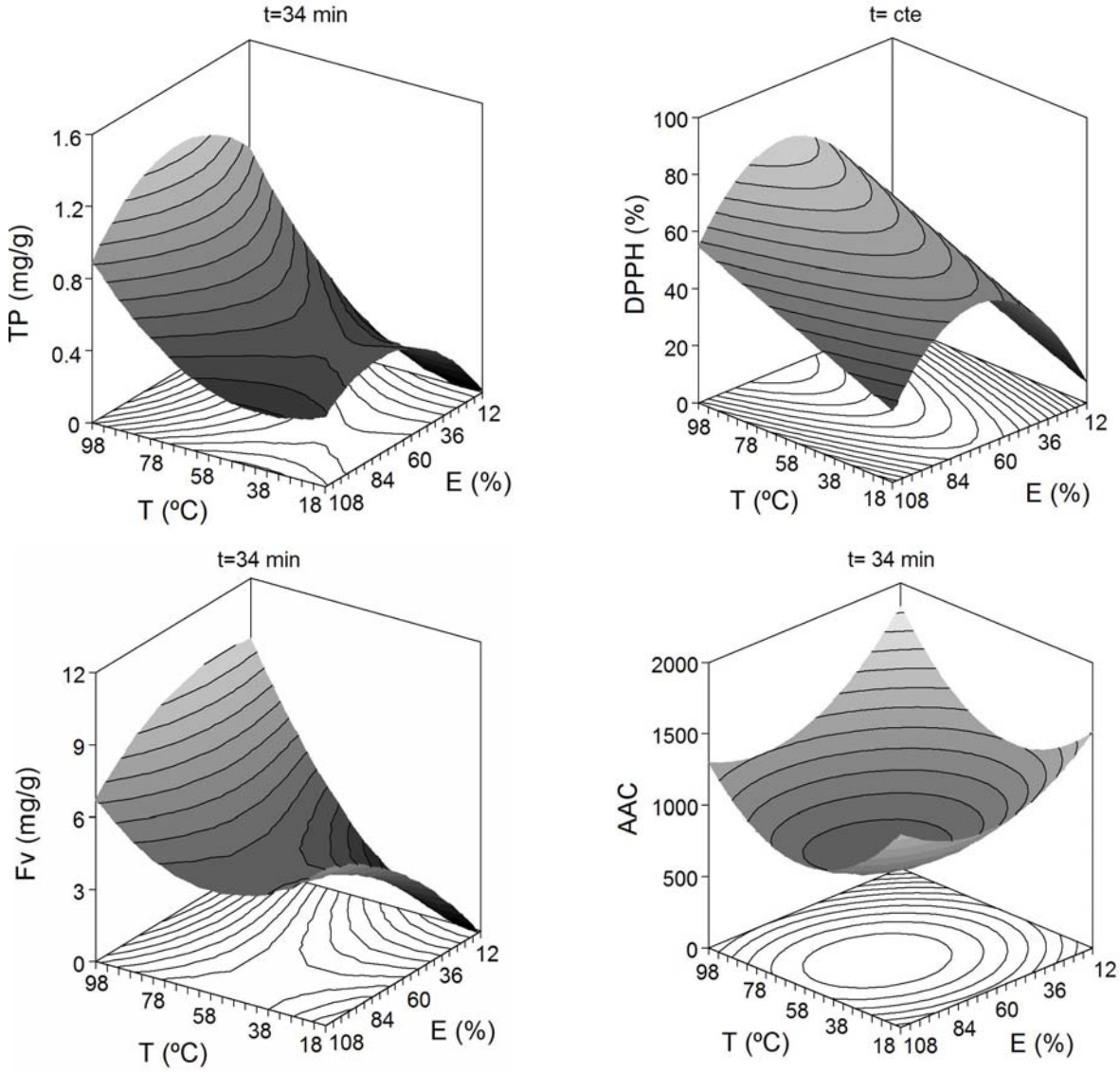
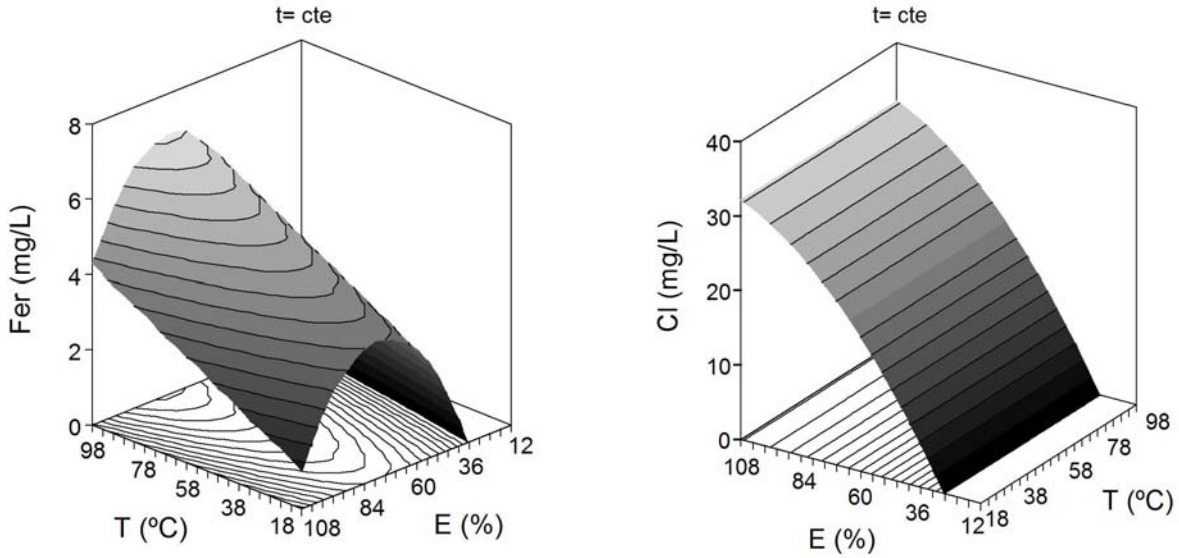
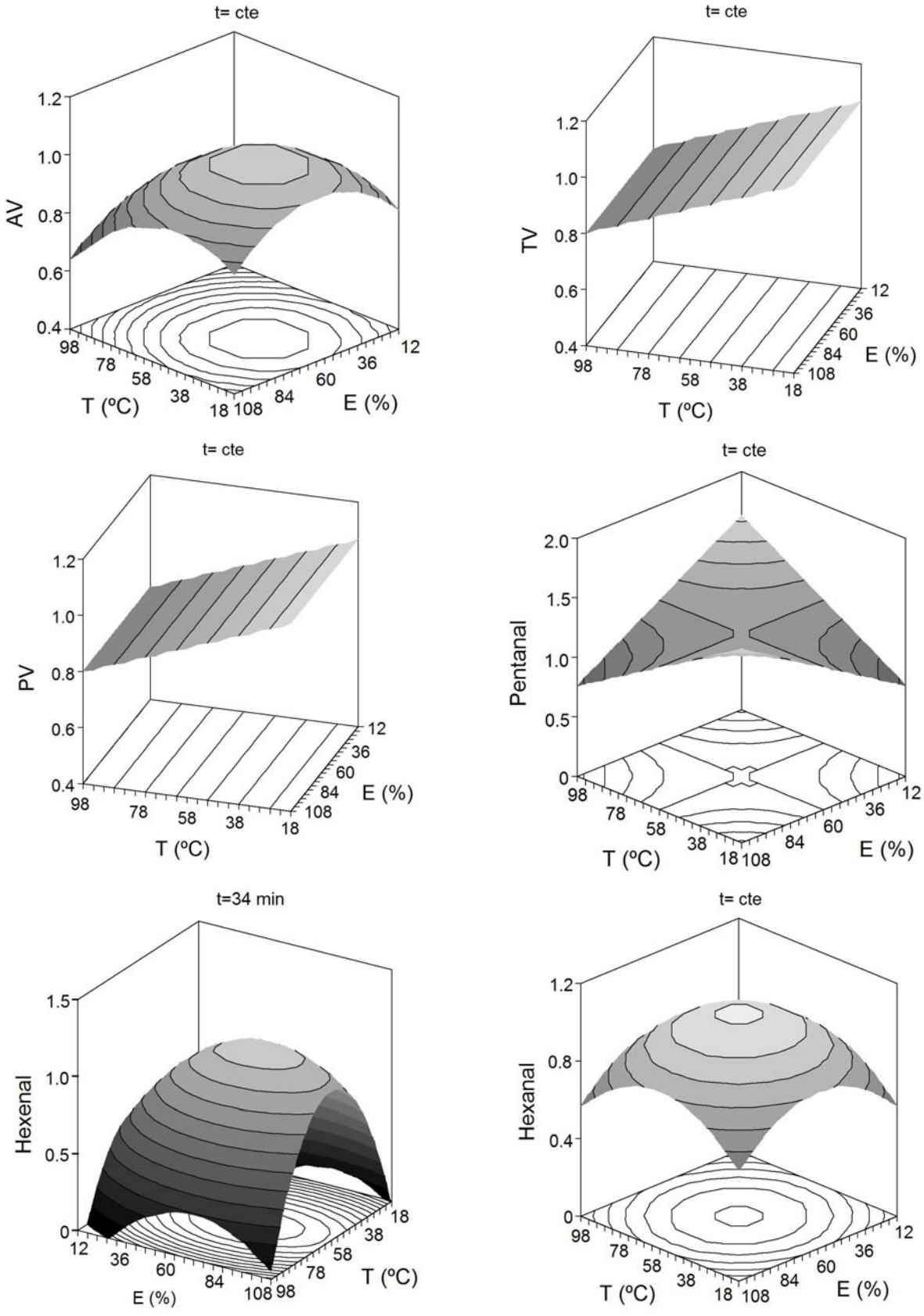

t=34 min
TP (mg/g)
1.6
1.2
0.8
0.4
0
98
78
58
38
18
T (ºC)
108
84
60
36
12
E (%)
t= cte
DPPH (%)
100
80
60
40
20
0
98
78
58
38
18
T (ºC)
108
84
60
36
12
E (%)
t=34 min
Fv (mg/g)
12
9
6
3
0
98
78
58
38
18
T (ºC)
108
84
60
36
12
E (%)
t= 34 min
AAC
2000
1500
1000
500
0
98
78
58
38
18
T (ºC)
108
84
60
36
12
E (%)

FIGURE 2


t= cte
Fer (mg/L)
0
2
4
6
8
T (ºC)
98
78
58
38
18
E (%)
108
84
60
36
12
t= cte
Cl (mg/L)
0
10
20
30
40
E (%)
108
84
60
36
12
T (ºC)
18
38
58
78
98

845 **FIGURE 3**


t= cte
AV
1.2
1.0
0.8
0.6
0.4
98
78
58
38
18
T (ºC)
108
84
60
36
12
E (%)
t= cte
TV
1.2
1.0
0.8
0.6
0.4
98
78
58
38
18
T (ºC)
12
36
60
84
108
E (%)
t= cte
PV
1.2
1.0
0.8
0.6
0.4
98
78
58
38
18
T (ºC)
12
36
60
84
108
E (%)
t= cte
Pentanal
2.0
1.5
1.0
0.5
0
98
78
58
38
18
T (ºC)
108
84
60
36
12
E (%)
t=34 min
Hexenal
1.5
1.0
0.5
0
12
36
60
84
108
E (%)
98
78
58
38
18
T (ºC)
t= cte
Hexanal
1.2
0.8
0.4
0
98
78
58
38
18
T (ºC)
108
84
60
36
12
E (%)

TABLES

Table 1

Independent variables			Y (mg/g)		DPPH (%)		TP (mg/g)		Fv (mg/g)		AAC		Fer (mg/L)		CI (mg/L)	
X_1: T	X_2: E	X_3: t	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p	R_{exp}	R_p
-1 (38.2)	-1 (36.2)	-1 (34.4)	95.09	98.94	42.40	44.35	0.34	0.34	2.72	2.43	1009.3	952.2	1.52	1.53	2.14	5.58
1 (76.8)	-1 (36.2)	-1 (34.4)	27.33	43.30	62.97	58.99	0.80	0.77	6.84	6.07	1091.9	1064.0	1.15	3.15	2.24	5.58
-1 (38.2)	1 (83.8)	-1 (34.4)	34.70	31.63	63.62	53.50	0.50	0.47	4.77	4.59	847.3	824.5	3.71	4.21	21.37	26.79
1 (76.8)	1 (83.8)	-1 (34.4)	42.56	51.60	75.11	68.14	0.84	0.78	6.54	5.88	895.7	841.8	5.20	5.83	29.55	26.79
-1 (38.2)	-1 (36.2)	1 (120.6)	100.60	97.35	40.25	44.35	0.41	0.41	3.11	2.94	1013.5	966.7	1.85	1.53	2.80	5.58
1 (76.8)	-1 (36.2)	1 (120.6)	104.00	112.86	60.92	58.99	1.08	1.05	8.63	7.98	1281.7	1203.8	0.36	3.15	4.20	5.58
-1 (38.2)	1 (83.8)	1 (120.6)	31.67	21.49	60.29	53.50	0.49	0.46	4.56	4.49	832.9	760.1	4.45	4.21	29.55	26.79
1 (76.8)	1 (83.8)	1 (120.6)	52.27	54.20	71.77	68.14	0.92	0.86	6.45	5.91	946.3	902.7	6.41	5.83	31.94	26.79
-1.68 (25)	0 (60)	0 (77.5)	56.97	67.28	51.42	52.38	0.45	0.46	3.73	3.75	880.3	950.4	2.08	3.56	14.01	17.90
1.68 (90)	0 (60)	0 (77.5)	91.03	72.55	72.68	77.00	1.13	1.21	7.38	8.54	1092.1	1164.4	7.71	6.28	20.33	17.90
0 (57.5)	-1.68 (20)	0 (77.5)	111.14	98.82	37.92	33.11	0.39	0.42	2.69	3.41	1044.2	1120.4	0.92	-0.84	0.56	-4.80
0 (57.5)	1.68 (100)	0 (77.5)	13.29	17.44	36.85	48.49	0.36	0.42	3.56	4.02	693.6	759.8	2.97	3.68	26.59	30.87
0 (57.5)	0 (60)	-1.68 (5)	64.08	51.54	62.18	64.69	0.55	0.59	3.94	4.67	705.2	752.7	4.59	4.92	18.63	17.90
0 (57.5)	0 (60)	1.68 (150)	72.59	76.95	64.49	64.69	0.74	0.77	5.20	5.65	721.3	816.1	6.08	4.92	18.89	17.90
0 (57.5)	0 (60)	0 (77.5)	67.91	67.02	67.34	64.69	0.63	0.63	4.80	4.52	698.6	725.9	5.38	4.92	17.85	17.90
0 (57.5)	0 (60)	0 (77.5)	65.59	67.02	68.40	64.69	0.62	0.63	4.49	4.52	735.3	725.9	5.34	4.92	19.59	17.90
0 (57.5)	0 (60)	0 (77.5)	67.14	67.02	59.96	64.69	0.64	0.63	4.58	4.52	720.5	725.9	3.85	4.92	15.37	17.90
0 (57.5)	0 (60)	0 (77.5)	67.80	67.02	63.15	64.69	0.64	0.63	4.45	4.52	742.9	725.9	5.85	4.92	19.76	17.90
0 (57.5)	0 (60)	0 (77.5)	64.40	67.02	64.89	64.69	0.63	0.63	4.34	4.52	743.5	725.9	3.62	4.92	14.29	17.90
0 (57.5)	0 (60)	0 (77.5)	67.86	67.02	65.56	64.69	0.65	0.63	4.64	4.52	739.2	725.9	5.29	4.92	20.37	17.90

Codification: $V_c=(V_n-V_0)/\Delta V_n$; Decodification: $V_n= V_0+(\Delta V_n \times V_c)$; V_n=natural value in the centre of the domain; ΔV_n= increment of V_n per unit of V_c.

Table 2

Parameters	Y (mg/g)	DPPH (%)	TP (mg/g)	Fv (mg/g)	AAC	Fer (mg/L)	CI (mg/L)
b_0 (intercept)	67.02	64.69	0.63	4.52	725.9	4.90	17.90
b_1 (T)	1.57	7.32	0.22	1.42	63.6	0.81	NS
b_2 (E)	-24.19	4.57	NS	0.18	-107.2	1.34	10.60
b_3 (t)	7.55	NS	0.05	0.29	18.8	NS	NS
b_{12} (TxE)	11.60	NS	-0.05	-0.75	-23.6	NS	NS
b_{13} (Txt)	10.49	NS	0.04	0.19	31.3	NS	NS
b_{23} (Ext)	-9.44	NS	-0.04	-0.31	-19.7	NS	NS
b_{123} (TxExt)	-7.30	NS	-0.01	-0.16	NS	NS	NS
b_{11} (T^2)	1.02	NS	0.07	0.58	117.2	NS	NS
b_{22} (E^2)	-3.14	-8,44	-0.08	-0.28	75.7	-1.24	-1.72
b_{33} (t^2)	-0.98	NS	0.02	0.23	20.7	NS	NS
R^2_{adj}	0.786	0.776	0.954	0.782	0.805	0.681	0.872
F1	7.99 $[F_9^{10} = 3.14] \Rightarrow S$	22.97 $[F_{16}^{3} = 3.24] \Rightarrow S$	44.63 $[F_{10}^{9} = 3.02] \Rightarrow S$	7.83 $[F_9^{10} = 3.14] \Rightarrow S$	9.70 $[F_{10}^{9} = 3.02] \Rightarrow S$	11.40 $[F_{16}^{3} = 3.24] \Rightarrow S$	65.47 $[F_{17}^{2} = 3.59] \Rightarrow S$
F2	0.86 $[F_{10}^{13} = 2.89] \Rightarrow S$	0.28 $[F_3^{13} = 8.73] \Rightarrow S$	0.71 $[F_9^{13} = 3.05] \Rightarrow S$	0.86 $[F_{10}^{13} = 2.89] \Rightarrow S$	0.77 $[F_9^{13} = 3.05] \Rightarrow S$	0.32 $[F_3^{13} = 8.73] \Rightarrow S$	0.17 $[F_2^{13} = 19.42] \Rightarrow S$
F3	84.84 $[F_6^{9} = 4.10] \Rightarrow NS$	3.92 $[F_6^{16} = 3.92] \Rightarrow S$	20.52 $[F_6^{10} = 4.06] \Rightarrow NS$	24.89 $[F_6^{9} = 4.10] \Rightarrow NS$	21.97 $[F_6^{10} = 4.06] \Rightarrow NS$	2.34 $[F_6^{16} = 3.92] \Rightarrow S$	2.27 $[F_6^{17} = 3.91] \Rightarrow S$

Table 3

Solanum tuberosum varieties	Extraction method	Fixed variables and solvents	TP mg/g peel	References
Russett Burbank (brown)	Microwave-assisted extraction	Time, methanol and microwave power	1.2-3.9 mg GAE/g dry potato	Singh et al. (2011)
Sava (brown)	Conventional solid/liquid extraction	Ethanol Water	68.7 mg GAE/100 g db 26.1 mg GAE/100 g db	Farvin et al. (2012)
Lady Claire (cream)	Pressurised liquids and solid/liquid extraction	Time and ethanol	409 mg/100 g db 431 mg/100 g db	Wijngaard et al. (2012)
Diamond (white)	Conventional solid/liquid extraction	Ethanol, methanol, acetone and hexane	1.12-2.91 mg GAE/g db	Mohdaly et al. (2013)
Red	Subcritical water extraction	Temperature, methanol and ethanol	46.4 mg GAE/100 g db	Singh & Saldaña (2011)
Agria (white)	Conventional solid/liquid extraction	Ethanol (36.2-100 %, v/v)	3.2-10.3 mg/100 g db	This study

Table 4

Parameters	*AV*	*PV*	*TV*	Pentanal	Hexanal	Hexenal
b_0 (intercept)	1.01	0.93	0.94	1.20	1.05	1.14
b_1 (*T*)	-0.04	-0.07	-0.07	NS	NS	0.18
b_2 (*E*)	NS	NS	NS	NS	NS	NS
b_3 (*t*)	NS	NS	NS	NS	NS	NS
b_{12} (*T*x*E*)	NS	NS	NS	0.11	NS	0.11
b_{13} (*T*x*t*)	NS	NS	NS	NS	NS	0.13
b_{23} (*E*x*t*)	NS	NS	NS	NS	NS	-0.11
b_{123} (*T*x*E*x*t*)	NS	NS	NS	NS	NS	0.12
b_{11} (T^2)	-0.03	NS	NS	NS	-0.06	-0.24
b_{22} (E^2)	-0.04	NS	NS	NS	-0.06	NS
b_{33} (t^2)	NS	NS	NS	NS	NS	-0.10
R^2_{adj}	0.550	0.414	0.416	0.184	0.418	0.810
F1	8.73 $[F^3_{16} = 3.24] \Rightarrow S$	14.43 $[F^1_{18} = 4.41] \Rightarrow S$	14.51 $[F^1_{18} = 4.41] \Rightarrow S$	5.27 $[F^1_{18} = 4.41] \Rightarrow S$	7.83 $[F^2_{17} = 3.59] \Rightarrow S$	12.61 $[F^7_{12} = 2.91] \Rightarrow S$
F2	0.32 $[F^{13}_3 = 8.73] \Rightarrow S$	0.13 $[F^{13}_1 = 244.7] \Rightarrow S$	0.14 $[F^{13}_1 = 244.7] \Rightarrow S$	0.31 $[F^{13}_1 = 244.7] \Rightarrow S$	0.259 $[F^{13}_2 = 19.42] \Rightarrow S$	0.60 $[F^{13}_7 = 3.55] \Rightarrow S$
F3	1.11 $[F^{16}_6 = 3.92] \Rightarrow S$	0.82 $[F^{18}_6 = 3.90] \Rightarrow S$	0.84 $[F^{18}_6 = 3.90] \Rightarrow S$	2.84 $[F^{18}_6 = 3.90] \Rightarrow S$	0.958 $[F^{17}_6 = 3.91] \Rightarrow S$	2.64 $[F^{12}_6 = 4.00] \Rightarrow S$